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Comparison of rice bran protein through different methods of extraction

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ABSTRACT

Protein content yield were determined and compared with three stages of different extraction treatments in defatted rice bran. These were : Single Stage process (ST), Double Stage process (DT), Multistage Process (MT) and pretreatment (PT). Results showed that the average amounts of protein ranged from 15.17g% to 25.36 g% in different treatments and reached the highest in yeast fermentation treatment. Thus, these indicated that yeast fermentation should be recommended to improve the protein content of extraction of protein from defatted rice bran.

Keywords: Defatted rice bran – different stages of treatments-Protein extraction-protein content

Introduction

Rice (*Oryza sativa L.*) is a major crop in the developing world. India is the world second largest producer of rice next to china. The world rice production in 2021 was approximately 503.17 million metric tonnes (USDA, 2021). India is the world's second largest producer of rice and it is the most important cereal food crop in the country. The Production of rice has increased by 2.39 million tonnes than the production of 118.43 million tonnes during 2020-21 (DAC&FW, 2020-21). West Bangal, Uttar Pradesh, Punjab, Andhra Pradesh, Odisha, Telangana, Chattisgarh, Tamil Nadu, Bihar and Assam are the major states contributing to the total rice production in India. West Bengal (15.57 MT), Uttar Pradesh (15.52MT) and Punjab (11.78MT) are three largest rice producing states in India (Economic survey 2020-21). In Tamil Nadu paddy was cultivated in an area of 18.75 lakh hectares with a production of 75.00 lakh metric tonnes and productivity of 4000 kg/ha at

2020-21 (Department of Economics and Statistics, 2020-21). The rice bran production in India is 14 to 15 MMT in 2020-21 out of which around 1.45-1.85 MMT goes to the feed sector. It is estimated that approximately 10-15% used in aqua feed, 30-35% in poultry and the rest in cattle feed industries. (Trade source & ministry of agriculture, 2020-21). Rice bran is an underutilized by products of rice milling industries. It is rich in nutrients which are moisture, carbohydrate, protein, fat, ash and crude fibre. The deactivation of lipase enzyme in rice bran is the best technique for rice bran consumption. The deactivation was done by stabilization method. It is a valuable method for rice bran utilization. Fermentation of wheat bran with baker's yeast enhanced protein extraction and functionality (Katina *et al*., 2012). There is paucity of information in literature on optimization of the fermentation conditions enhanced by the use of baker's yeast for extraction of protein concentrate (Chinma *et al*., 2014).

The potential of rice bran for improving human health was envisaged by the researchers worldwide and reported that rice bran could be used as ingredient in food products mainly to improve the nutritional, functional and therapeutic values. Studies indicated that foods supplemented with rice bran proteins helps in reduction of bone loss in women suffering from postmenopausal osteoporosis and found that many nutraceuticals developed from rice bran protein possess hypolipidemic, anti tumor, antioxidant, ergogenic and laxative properties. The present work was aimed to study the extraction process of protein using different stages of treatments from defatted rice bran.

2. Materials and methods

2.1 Purchase of raw material

Defatted rice bran was purchased from Vagai Agro Products limited, Nagari Sholavandan road, Thanichiyam village, vadipatti taluk, Madurai, Tamil Nadu, India. Chemical reagents for Nitrogen determination were purchased from Super Scientific Suppliers, 87, Spencer compound, Near R.R. Travels, Bus stand south side, Dindigul-624003. Baker's Yeast was purchased from Sona departmental store, Chinnalapatti, Dindigul.

2.2 Extraction process of protein using different stages of treatments

To compare the protein content from defatted rice bran, three stages of treatments were designed. The extraction was performed using defatted rice bran. Blending, waterbath shaking, addition of KMS, enzyme (alpha amylase) and Baker's Yeast were use to extract the soluble dry matter from the defatted rice bran: The blending time (60 sec), heating temperature 80^{o}C time 10 min, and fermentation time were fixed at of the extraction treatment. Each suspension was then centrifuge at level 2 for 20 min, and the resulting supernatant was filtered. The spent solid was cabinet dried and stored at ambient temperature until further analyse.

2.2.1. Extractability of protein from defatted rice bran by single stage process

Five different treatments were applied in single stage (SS) process. In the first treatment (SST1) rice bran was heated at 80^{o}C for 10 min, for the second treatment (SST2) was alkali treatment pH was adjusted to

10 with 1N NaoH, for the third treatment (SST3) was treated with enzyme (alpha amylase) at the ratio of 1:100 and pH was adjusted with 6.25 using 0.1N HCL for 3.5hrs incubation. For the fourth treatment (SST4) was natural fermentation for 20hrs and the treatment five (SST5) was yeast fermentation with 3% baker's yeast for 20 hrs.

2.2.2. Extractability of protein from defatted rice bran by double stage process

Five different treatments were applied in double stage (DS) process. In the first treatment (DST1) rice bran was alkali treatment pH was adjusted to 10 with 1N NaOH followed by heated at 80^{o}C for 10 min. For the second treatment (DST2) was treated with enzyme (alpha amylase) at the ratio of 1:100 and pH was adjusted with 6.25 using 0.1N HCL for 3.5hrs incubation followed by heated at 80^{o}C for 10 min. For the third treatment (DST3) was treated with enzyme (alpha amylase) at the ratio of 1:100 and pH was adjusted with 6.25 using 0.1N HCL for 3.5hrs incubation followed by alkali treatment. For the fourth treatment (DST4) was heated at 80^{o}C for 10 min followed by natural fermentation for 20hrs and the treatment five (DST5) was heated at 80^{o}C for 10 min followed by yeast fermentation with 3% baker's yeast for 20 hrs.

2.2.3. Extractability of protein from defatted rice bran by multi-stage process

Three different treatments were applied in multi stage (MS) process. In the first treatment (MST1) was treated with enzyme (alpha amylase) at the ratio of 1:100 and pH was adjusted with 6.25 using 0.1N HCL for 3.5hrs incubation followed by alkali treatment and heated at 80^{o}C for 10 min. For the second treatment (MST2) was treated with enzyme (alpha amylase) at the ratio of 1:100 and pH was adjusted with 6.25 using 0.1N HCL for 3.5hrs incubation followed by heated at 80^{o}C for 10 min and yeast fermentation. For the third treatment (MST3) was treated with yeast fermentation with 3% bakers yeast for 20 hrs followed by enzymatic hydrolysis and heating.

2.2.4. Pretreatments of defatted rice bran

The pretreatment (PT) methods were adapted from Tang *et al.* (2003) and Chinma *et al.* (2014) the same amount of rice bran weighting approximately 10 g was used for all treatments. Untreated Defatted rice bran was control (PTT0). The five pretreatment methods are shown in table 4. The first pretreatment (PTT1) method consisted of enzyme treatment. The second pretreatment (PTT2) was fermentation of rice bran with 3 % baker's yeast. Third pretreatment (PTT3) was fermentation of rice bran with 15 % baker's yeast. Fourth pretreatment (PTT4) was fermentation of rice bran with 30 % baker's yeast and fifth pretreatment (PTT5) consisted of enzyme and fermentation of rice bran.

2.3 Estimation of Protein

Protein was analzed by the amount of nitrogen available in the sample by micro kjeldhal method. 200 mg of sample was transferred in to 250 ml digestion flask along with four gram of catalytic mixture (sodium sulphate and copper sulphate) 5:1 ratio and 10 ml concentrated sulphuric acid was added to the sample fixed to the digestion unit (420^{o}C) and wait for two hours until a clear solution was obtained.

The digestion sample was placed in the distillation unit for ammonia recovery. The solution was

distilled and the ammonia was collected in the reciever solution. The solution was titrated against 0.1N HCL for the end point, until the color changes. The sample procedure was repeated to get the blank value and the nitrogen content of the sample were calculated. The nitrogen value multiplied by factor 6.25 gives the crude protein content of the sample in per cent.

Calculation :

$$\text{Nitrogen (\%)} = \frac{\text{Sample titre value - blank titre value x equivalent of nitrogen (14.01) x normality of HCL (0.1)}}{\text{Weight of the sample x 1000}} \times 100$$

Protein (%) = Nitrogen (%) x conversion factor (6.25)

2.4 Statistical analysis

Analysis of variance and significant difference among means were calculated by One way ANOVA. Data were analyzed using Data Entry Module for Agres Statistical Software (Version 3.01). The data obtained from the various experiments were subjected to statistical analysis to find out the impact of different treatments by using Factorial Completely Randomized Design (FCRD) method as described by Cochran and Cox (1957). P values <0.05 were considered as statistically significant.

3. Results and Discussion

3.1 Extractability of protein from defatted rice bran by single stage process

Table 1 and Fig 1 shows the effect of treatment on the yield, protein content of extract and residue from defatted rice bran in single stage process. The residue yield of single stage process were SST1 (69.80%), SST2 (50.64%), SST3 68.49%, SST4 50.14% and SST5 65.4% respectively. On the other hand extract yield of single stage process were ranged from 6.20 % to 18.14%. The protein contents in the extract were less than 21% and protein contents in the residue were ranged from 12.56% to 22.20%.

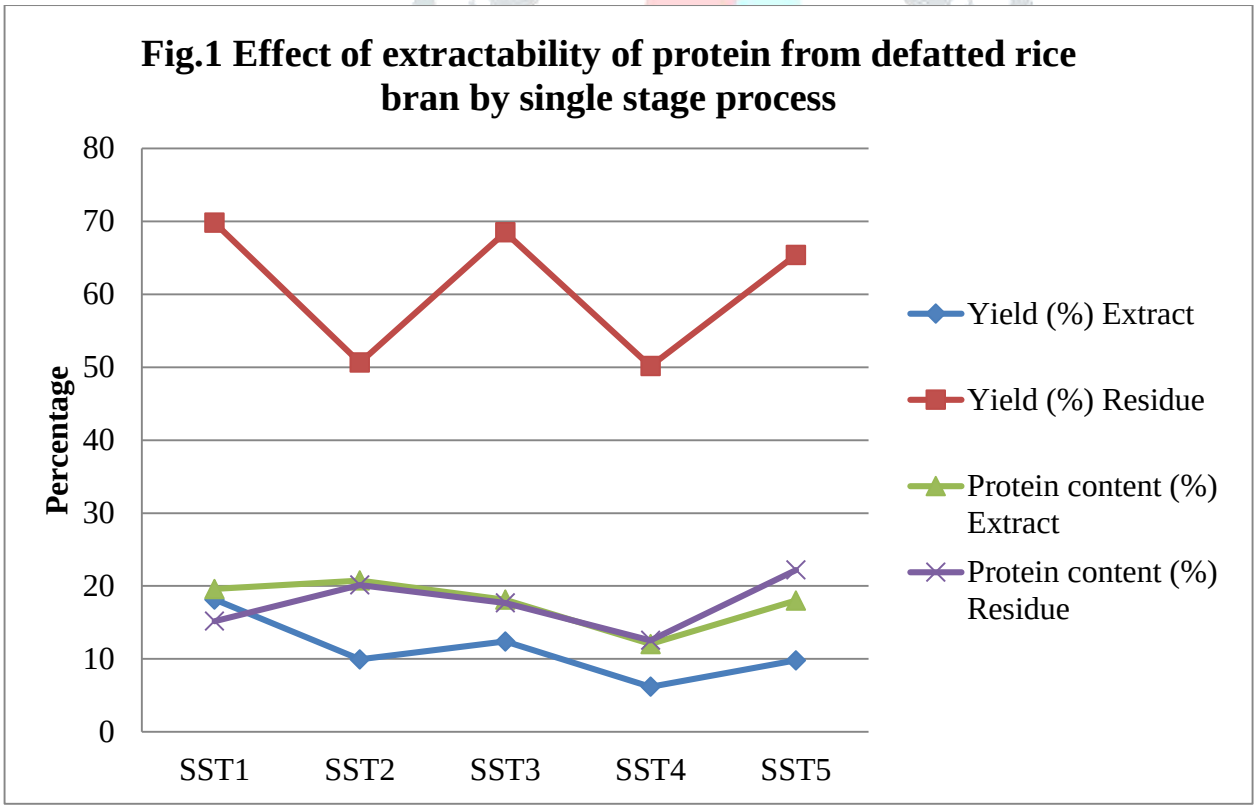
Theerakulkait *et al.* (2006) reported that protein extractability was 44.4% in total protein in rice bran used for alkali extraction.

Khuwijitjaru *et al.* (2007) evaluated & result indicated that increasing treatment temperatures increased the protein content and time treatment was affected the protein content.

Table 1. Extractability of protein from defatted rice bran by single stage process

S.No.	Treatments	Protein concentrate			
		Yield (%)		Protein content (%)	
		Extract	Residue	Extract	Residue
1.	SST1	18.14 ± 0.54	69.80 ± 2.09	19.60 ± 0.59	15.18 ± 1.35
2.	SST2	09.95 ± 0.20	50.64 ± 1.01	20.75 ± 0.41	20.15 ± 0.4
3.	SST3	12.40 ± 0.37	68.49 ± 2.05	18.12 ± 0.54	17.68 ± 0.53
4.	SST4	6.20 ± 0.16	50.14 ± 1.32	12.04 ± 0.31	12.56 ± 0.33
5.	SST5	9.80 ± 0.35	65.4 ± 2.34	18.01 ± 0.64	25.36 ± 0.80

	Yield		*Protein content*	
	SED	*CD (0.05)*	*SED*	*CD (0.05)*
Y	0.48258	1.00665 **	0.24119	0.50312 NS
T	0.76303	1.59166 **	0.38136	0.79550 **
YT	1.07909	2.25094 **	0.53932	1.12501 **



3.2 Extractability of protein from defatted rice bran by double stage process

After the single stage process the effect of a additional treatment was investigated the results, as also shown in Table 2 indicate that higher yield for the achieved in DST4 residue and DST2 had highest protein

content of about 35.24% in DST2 residue. On the other hand extract yield were ranged from 4.6 % to 21.56%. The protein contents in the extract were ranged from 16.75% to 18.43%.

Jiamyangyuen *et al.* (2005) reported that using high pH for protein extraction could bring some disadvantages; for example, (1) protein denaturation and hydrolysis at high pH, resulting in undesirable flavor and odor, (2) increase Maillard reaction, leading to dark colored product, (3) decreased nutritive value of protein, especially essential amino acid such as lysine, and (4) increased extraction of non-protein component, which also coprecipitated with protein and lower protein purity. At higher pH, some non-protein nitrogen may be a component accounting for high yield.

Yadav *et al*. (2012) reported that the maximum yield of 13.2 per cent for RBPC was obtained at alkaline pH of 11, temperature of 60ºC and extraction time of 60 minutes.

Table 2. Extractability of protein from defatted rice bran by double stage process

S.No.	Treatments	Protein concentrate			
		Yield (%)		Protein content (%)	
		Extract	Residue	Extract	Residue
1.	DST1	21.56 ±0.65	66.53 ± 2.0	18.43 ± 0.55	19.25 ± 0.58
2.	DST2	11.33 ± 0.23	66.67 ± 1.33	16.75 ± 0.44	35.24 ± 0.7
3.	DST3	12.60 ± 0.38	50.45 ± 1.51	17.48 ± 0.62	33.60 ± 1.01
4.	DST4	10.00 ± 0.26	73.60 ± 1.94	18.43 ± 0.66	17.25 ± 0.45
5.	DST5	4.6 ± 0.16	63.01 ± 2.32	17.25 ± 0.78	20.31 ± 0.73

	Yield		*Protein content*	
	SED	*CD (0.05)*	*SED*	*CD (0.05)*
Y	0.48959	1.02127**	0.24629	0.51375**
T	0.77411	1.61477**	0.38942	0.81231**
YT	1.09476	2.28363**	0.55072	1.14879**

3.3 Extractability of protein from defatted rice bran by multi stage process

As shown in Table 3, residue yield was upto 76.50% and extract yield was 21%. The protein content of MST1 had highest 33.57% compare to other treatments.

Table 3. Extractability of protein from defatted rice bran by multi-stage process

S.No.	Treatments	Protein concentrate			
		Yield (%)		Protein content (%)	
		Extract	Residue	Extract	Residue
1.	MST1	21.00 ± 0.63	47.75 ± 1.43	15.15 ± 0.45	33.57 ± 1.01
2.	MST2	9.45 ± 0.19	76.50 ± 1.53	16.18 ± 0.32	16.26 ± 0.33
3.	MST3	4.50 ± 0.13	63.15 ± 1.89	16.25 ± 0.49	18.20 ± 0.55

	Yield		*Protein content*	
	SED	***CD (0.05)***	***SED***	***CD (0.05)***
Y	**0.55808**	**1.21596****	**0.27056**	**0.58951****
T	**0.68350**	**1.48924****	**0.33137**	**0.72200****
YT	**0.96662**	**2.10610****	**0.46863**	**1.02106****

3.4. Effect of pretreatments on yield and protein content of defatted rice bran

Table 4 shows the effect of pretreatment on yield and protein content of defatted rice bran. The protein content of different pretreated product increased from 21.36% to 34.93%. the percent yield was highest in PTT2 89.80%. the lowest yield was obtained from PTT3 80.02%.

Hernandez *et al.* (2000) investigated that the from the original protein in the bran, 21.5% is solubilized in the first reaction during α-amylase treatment.

Defatted rice bran fermented with yeast to afford a food product having superior prebiotic for probiotic composition enrichment. Fermentation of Defatted rice bran with yeast yeids a prebiotic composition that can promote the growth of beneficial intestinal bacteria (probiotic).

The prebiotic and probiotic compositions release substances that have desirable health effects upon consumption. The fermented and dried Defatted rice bran with reduced particle size has desirable health benefits when consumed (Hettiarachchy Navam, 2009).

The protein production, especially the single cell protein is more in Defatted rice bran due to the bioconversion of cellulose in Defatted rice bran (Ravinder *et al.,* 2003).

Chinma *et al.* (2014) optimized the conditions for yeast pretreatment of rice bran protein extraction were achieved at 30^{o} C for 17 h using 3% yeast concentration to obtain a protein yield of 23.37%, which showed no significant difference ($p \geq 0.05$) from the response surface methodology.

Table 4. Effect of pretreatments on yield and protein content of defatted rice bran

S.No.	**Treatments**	**Defatted rice bran**	
		Yield (%)	**Protein content (%)**
1.	PTT0	100.00	16.5
2.	PTT1	85.50 ± 1.71	34.93 ± 0.49
3.	PTT2	89.80 ± 2.69	21.36 ± 0.7
4.	PTT3	80.02 ± 2.11	25.68 ± 0.67
5.	PTT4	87.22 ± 2.78	28.68 ± 1.03
6.	PTT5	84.35 ± 2.53	24.50 ± 0.73
	SEd	2.0014	0.5980
	CD	4.4594**	1.3029 **

4. Conclusion

Yield of the protein concentrate prepared from extracts were ranged between 5 and 22 percent and residue yield ranged from 47 to 76 percent. Both extracts and residue showed high protein content ranged from 12 to 35 percent. Multi-stage extraction exhibited better yield and protein content of concentrate than other process. Pretreatment of defatted rice bran with enzyme and yeast had better yield (85%) and protein content (>20%) compared to multistage processes. The Fermented Defatted Rice Bran is an excellent source of dietary fibre for addition to food and it offers all the nutritional and neutraceutical benefits of whole grain. The use of baker's yeast in the fermentation of rice bran for extraction of protein concentrate is simple, and can be more effectively used to improve the protein extraction yield compared to natural fermented (20.31%) and untreated rice bran (16.25%). Defatted rice bran protein can be considered as a suitable diet for human consumption and has the potential to use in new food formulations.

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